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C-erb-b2 expression in luminal b breast cancer has higher axillary lymph node involvement ratio

C-erb-B2 expression in luminal-B breast cancer

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Abstract
Aim: In this study, the effect of c-erb-B-2 (HER-2/neu) expression on axillary lymph node involvement in Luminal- B breast cancer was examined.
Material and Methods: One hundred seven female patients were included in this study who were classified as Stage 1, Stage 2 and Stage 3 pathologically, with positive Estrogen (ER) and Progesterone Receptor (PR) and with the diagnosis of invasive ductal carcinoma with a single focal mass and not a synchronous tumour and received surgical therapy. The lymph node involvement ratio (LNIR) was divided into two groups as over and below 25%. These acquired data were compared with the groups with positive and negative c-erbB-2 gene expression, axillary LNI status, and LNIR separately.
Results: The data of 107 female patients aged between 27 and 87 years were evaluated in this study. The mean age of the patients was 55.69±12.68 years. LNIR was found to be less than 25% in 76.6% (n: 82) of the patients, and over 25% in 23.4% (n: 25) of the patients. The c-erbB-2 positivity was significantly different in the axilla with and without metastatic lymphadenopathy (p =0.026). There was no statistically significant relationship between tumour diameter and metastatic lymphadenopathy. Although axillary metastatic lymphadenopathy positivity was found to be significant in patients with lymphovascular invasion (p<0.001), it was also found to be significant, as in the group with LNIR>25 (p<0.001).
Discussion: This study demonstrated that high expression of c-erbB-2 is associated with poor prognosis of breast cancer by increasing axillary LNI. In hormone (Estrogen and Progesterone) positive patients, if c-erB-2 is also positive, there is greater number of axillary LNI.

Keywords
Breast Cancer, Axillary Lymph Node, Involvement

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Introduction

Breast cancer is the most common cancer among women in Turkey and in the world, and ranks second in cancer deaths after lung cancer. It is considered that one out of eight women will develop invasive breast cancer during their lifetime. This rate is higher in patients with a family history and those with breast cancer-related gene mutations [1]. Breast carcinomas are tumours of different groups with diverse characteristics in terms of histopathological features, hormone receptor levels, clinical and treatment response. The lymph node involvement ratio (LNIR), which indicates the ratio of positive lymph nodes to the total lymph nodes removed, is suggested as an alternative prognostic factor because it offers a more accurate prognostic grouping opportunity and is less influenced by dissection width. C-erbB-2 (HER-2/neu) oncogene is in the form of a single copy in non-pathological cells and is located on chromosome 17. When overexpressed in breast cancer, c-erbB-2 enhances invasion and metastasis, strengthening growth and proliferation [2]. In this study, we aimed to investigate the effect of the increase of c-erbB-2 expression on axillary lymph node involvement and to determine the factors causing axillary involvement, which has the greatest impact on prognosis in patients with invasive ductal carcinoma.

Material and Methods

This study was performed on patients who were admitted to our clinic with the diagnosis of breast cancer and underwent surgical treatment. Of the 309 female breast cancer patients operated between June 2012 and January 2016, 107 patients who met the criteria were included in the study. Among these patients, only Stage 1, Stage 2 and Stage 3 female patients with pathological diagnosis of invasive ductal carcinoma with a single focal mass and not a synchronous tumor, and with positive Estrogen Receptor (ER) and Progesterone Receptor (PR) were included. Information about the patient's clinical stage and distant metastasis was obtained from the patient files and data processing system in the General Surgery Polyclinic of our hospital, and patients who received neoadjuvant treatment before surgery were not included in the study. From the patient files, ages of patients, histological type, diameter, hormone receptor status, c-erbB-2 expression status (according to immunohistochemical scoring), lymphovascular invasion status of the tumour, localization, axillary lymph node involvement, histological grade of the tumor were determined from the pathology reports.

The cases in the file records were divided into 3 groups according to the tumor size as 2 cm and under, 2 to 5 cm and 5 cm and above reached as a result of pathological examination. Tumor location was divided into 5 quadrants as upper outer quadrant, upper inner quadrant, lower outer quadrant, lower inner quadrant and retroareolar. The cases were divided into two groups as those with and without lymphovascular invasion. c-erbB-2 was divided into 2 groups as negative with score-0, score-1 if no membranous staining is observed in invasive tumour cells or incomplete, indistinct membrane staining is present in cells of 10% or less and positive with values above (score-2, score-3) according to the immunohistochemical (IHC) examination. Two groups were created as the dependent variable; groups with axillary lymph node metastasis and above were identified as positive and those without any metastasis were identified as negative. Tumors staining only -3 positives in IHC were accepted as HER-2 positive. In addition, LNIR, which is frequently used recently and also in the prognosis evaluation with more precision, was divided into two groups as over and below 25%. We used LNIR because of more sensitive prognostic grouping and less effect on dissection width. For LNRI, the purpose of grouping as 25% below and above is to apply the findings according to the percentile values where the cases are partially homogeneously distributed to the groups and transferring the findings to be only 25% below and above for ease of expression since both grouping methods are statistically the same results. Sentinal lymph node sampling was performed in all patients. Patients who were negative as a result of sentinal sampling were included in the group with LNIR less than 25%. Axillary lymph node dissection in patients who were positive for sentinel sampling was completed and LNIR calculation was made according to the pathology result. LNIR is the lymph node involvement rate that expresses the ratio of positive lymph nodes to the total lymph nodes removed.

[LNIR = (positive lymphadenopathy / total number of lymph nodes removed) x 100]

All these acquired data were compared with the groups with positive and negative c-erbB-2 gene expression, axillary lymph node involvement status and LNIR separately. In addition, histological grade, lymphovascular invasion, tumor diameter data, axillary lymph node involvement status and LNIR were compared separately. Immunohistochemical (immunoperoxidase-code: A0485, C-erbB-2 oncoprotein, kid: DAKO LSAB (R) 2 kit-KO675) analysis was performed as a pathological method for c-erbB-2. Values of 50% and above were considered positive in terms of c-erbB-2. Accordingly, those with a fluorescence detection rate of 50% or more were considered c-erbB-2 positive and used within the luminal group classification. Cases in our study consisted of the Luminal B group (Luminal B is considered as ER and/or PR positive, c-erb-B2 negative or positive and Ki-67 proliferation index high patients).

Statistical Analysis

Compliance of quantitative data with normal distribution of the numerical variables were evaluated using the Shapiro-Wilk test, normality test and QQ graphs, and parametric methods were used in the analysis of variables with normal distribution, non-parametric methods were used in the analysis of variables which did not have a normal distribution. Pearson Chi-Square and Fisher's Exact tests were used to compare categorical data. Quantitative data were expressed as average ± std values in the tables, and categorical data were expressed as n and percentages. Data were analyzed at 95% confidence level and p-values greater than 0.05 were considered insignificant and p-values less than 0.05 were considered significant. $p < 0.05$ was considered statistically significant.

Ethical approval

The study was in line with the principles set out in the Declaration of Helsinki. All patients signed informed consent for their data to be used for research purposes after a clear and complete explanation and consent was recorded in the

patients' medical records. The Institutional Review Board of Yıldırım Beyazıt University, Ankara, Turkey approved this study (No: 06.09.2017-161).

Results

In this study, the data of 107 female patients aged between 27 and 87 years were evaluated. The mean age of the patients was 55.69±12.68 years. Fifty-nine (55.1%) tumours were located on the left and 48 (44.9%) were located on the right breast. Fifty-one (47.6%) tumours were in the upper outer quadrant, 16 (15%) in the lower outer quadrant, 17 (15.9%) in the upper inner quadrant, 14 (13%) in the lower inner quadrant, 9 (8.5%) were located in the retroareolar area; 68.2% (n:73) of the patients underwent Modified Radical Mastectomy (MRM) operation, 29.9% (n:32) had Sentinel Lymph Node (SLN) operation together with simple mastectomy or segmental mastectomy, 1.9% (n:2) had axillary dissection with segmental mastectomy. It was detected that in 25.2% (n:27) of patients the tumor size was less than 2 cm, in 58.9% (n:63), the tumor size was between 2-5 cm, and in 15.9% (n:17), the tumor size was over 5 cm. Grade 1 tumor was detected in 18.7% (n:20) of the patients, Grade 2 tumour in 59.8% (n:64) and Grade 3 tumour in 21.5% (n:23) of the patients. When those with score-0 and score-1 in immunohistochemical staining were grouped as negative and those with other scores were grouped as positive, 51.4% (n:55) of the patients were determined as c-erB-2 negative and 48.6% (n:52) were c-erB-2 positive.

According to the pathological TNM staging classification of breast cancer, 13.1% (n:14) of them were found to be Stage-1, 30.8% (n:33) of them Stage-2a, 27.1% (n:29) of them stage-2b, 15% (n:16) of them Stage-3a and 14% (n:15) of them were found to be Stage-3c. While metastatic lymphadenopathy was not detected in the axilla in 36.4% (n:39), metastatic lymphadenopathy was found in 63.6% (n:68) of the patient. LNIR was found to be less than 25% in 76.6% (n:82) of the patients, and over 25% in 23.4% (n:25) of the patients (Table 1). The c-erbB-2 positivity was significantly different in the axilla with and without metastatic lymphadenopathy (p = 0.026) (Table 2).

In the statistical study on whether tumour diameter has an effect on axillary lymph node involvement; the group with LNIR> 25 was found to be higher statistically among patients with tumor diameter of more than 5 cm in pathological measurements (p=0.008), no statistical relation between tumor diameter and metastatic lymphadenopathy was observed.

In the analysis of the effect of lymphovascular invasion on axillary lymph node involvement; although axillary metastatic lymphadenopathy positivity was found to be significant in patients with lymphovascular invasion (p <0.001), it was also found to be significant as in the group with LNIR> 25 (p <0.001) (Table 3). There was no statistically significant correlation between LNIR and c-erbB-2 positivity (p = 0.193) (Table 2).

Table 1. Axillary metastatic lymph node and LNIR status of the cases (n: 107)

		n	percent
Metastatic lymph node	Negative	39	36.4%
	Positive	68	63.6%
LNIR	<25%	82	76.6%
	>25%	25	23.4%

LNIR: Lymph node involvement ratio

Table 2. The association of c-erbB-2 expression with metastatic LAP and LNIR in axilla (n:107)

		c-erbB-2 expression		
		Negative	Positive	
Metastatic LAP	Negative	26	13	p=0.026
		47.3%	25.0%	
	Positive	29	39	
		52.7%	75.0%	
LNIR	<25%	45	37	p=0.193
		54.9%	45.1%	
	>25%	10	15	
		40%	60%	

LNIR: Lymph node involvement ratio, LAP: Lymphadenopathy

Table 3. The relationship between lymphovascular invasion and metastatic LAP and LNIR in axilla

		Lymphovascular invasion		
		Negative	Positive	
Metastatic LAP in axilla	Negative	32	7	p<0.001
		82%	18%	
	Positive	28	40	
		41.1%	58.9%	
LNIR	<25%	57	25	p<0.001
		69.5%	31.5%	
	>25%	3	22	
		12%	88%	

LNIR: Lymph node involvement ratio, LAP: Lymphadenopathy

Discussion

Various prognostic factors are used to determine the current clinical features and future high-risk group in breast cancer. The most important factor among these is whether the axillary lymph nodes contain metastases, and if so, the number of lymph nodes involved. Tumor diameter, histological grade, histological tumor type, presence of hormone receptor (ER, PR), rate of tumor proliferation (number of mitosis, S-phase reaction Ki-67 proliferation index) and molecular prognostic factors (c-erb-B2, onco-suppressor genes) are other prognostic factors. LNIR, which is suggested as an alternative prognostic factor, can be considered as a prognostic factor [3-6].

In recent years, the increase in recurrence rates, especially among women with negative axillary, has led researchers interested in breast cancer to study new markers, which are also called secondary prognostic factors. This new classification, called the molecular subgroup, was first proposed in 2000 by Perou et al. [7] in a comprehensive study showing differences in gene expression. Currently, breast cancers are initially classified into five groups as luminal A, luminal B c-erb-B2 (-) and c-erb-B2 (+), non-luminal c-erb-B2 (+), triple negative and Null type according to the applicable molecular classification. The subtypes, which are called luminal, are classified according to

ER and PR presence, c-erb-B2 amplification and overexpression Ki 67 proliferation index. Luminal A is considered ER and/or PR positive, c-erb-B2 negative Ki-67 proliferation index low (<15%); Luminal B c-erb-B2 (-) is considered ER and/or PR positive, c-erb-B2 negative Ki-67 proliferation index high (> 15%); Luminal B cerb-B2 (+) is considered ER and/or PR positive Ki-67 proliferation index low and/or high, cerb-B2 positive; non Luminal c-erb-B2 (+) is considered ER and PR are considered negative, c-erb-B2 positive, and Triple negative is considered ER, PR and c-erb-B2 negative. Morphological and molecular studies have shown that these subtypes with different hormonal profiles respond differently to treatment modalities and they are different in prognosis [8]. In a study by Voduc et al. [9] on 2985 patients with breast cancer, it was revealed that Luminal B group patients showed a poorer prognosis than Luminal A group. However, in the study by Chengshuai et al. [10] in 814 patients, axillary lymphadenopathy involvement was high in the Luminal-b c-erb-B2 + patient group. Our study is investigated whether c-erbB-2 can be used as a biological indicator showing axillary lymph node involvement in advance or enabling us to make a prediction.

C-erbB-2 (HER-2/neu) is a member of the epidermal growth factor receptor (EGFR) family. It is located on chromosome 17q and acts as a tyrosine kinase receptor protein. C-erbB-2 receptor positivity is seen in approximately 25-30% of invasive cancers. It was found in 50-60% of cases of ductal carcinoma in situ (DCIS). HER-2 positivity was found in 48.6% of the patients with invasive ductal cancer included in our study. There is no clear consensus on the effect of c-erbB-2 on lymph node involvement [11]. In a retrospective study by Tong et al. [12] on 316 women in 2017, it was detected that increased c-erbB-2 expression increased axillary lymph node involvement. In contrast, it was detected that 18.2% of the cases with SLN positive were HER-2 positive and 81.8% of them were HER-2 negative in the research by Nathanson et al. [13] on 1063 patients. In this study, it was detected that HER-2 positivity had a negative effect on axillary lymph node involvement. In our study, c-erbB-2 positivity was found to be significantly different in groups with and without metastatic lymphadenopathy in the axilla. Axillary lymph node involvement was found in 75% of the patients with c-erbB-2 positivity. No relationship between c-erbB-2 and LNIR was detected in the study by Akdur et al. [14] on 150 patients, which is one of the rare studies on the LNIR and c-erbB-2 relationship in the literature. In our study, no statistically significant relationship was found between c-erbB-2 positivity and LNIR.

Lymphovascular invasion is roughly referred to as invasion of tumour tissue and surrounding lymphatic and vascular structures. Lymphovascular invasion is observed in one third of breast cancer patients. The presence of lymphovascular invasion is a negative prognostic factor for locoregional recurrence [15,16]. It was found that peritumoral lymphovascular invasion is an independent risk factor for local recurrence and death in the cohort study of 1704 patients who did not receive any systemic adjuvant therapy [17]. However, it was revealed in the multicentre study by Ejlertsen B et al., published in 2009 [18] on 15.659 patients that when lymphovascular invasion accompanies other poor prognostic factors of breast cancer (tumor size, grade, lymph node involvement, ductal histology, hormone receptor positivity), it acts negatively on the prognosis of the patient but it does not have an effect on the prognosis of the patient by itself. In other words, the presence of lymphovascular invasion alone does not include low-stage breast cancer in the high-stage group. In a study by Ragage F et al. on 374 patients [19], lymphovascular invasion was found to increase axillary lymph node involvement. However, multivariate models were evaluated in this study and no comparison was made with other factors affecting prognosis. It was shown in the research by He KW et al. [20] on 255 patients that the presence of lymphovascular invasion increases axillary lymph node involvement when considered with other prognostic factors. In the study by Akdur et al. [14], it was found that LNIR tended to be higher in cases with lymphovascular invasion. In our study, a significant difference was found between axillary lymph node involvement in patients with lymphovascular invasion and axillary lymph node involvement in patients without lymphovascular invasion. Axillary involvement was not observed in 79.4% of patients without lymphovascular invasion, whereas it was observed in 60.3% of patients with lymphovascular invasion. This result showed that lymphovascular invasion increases lymph node involvement.

Conclusion

This study demonstrated that high expression of c-erbB-2 is associated with axillary lymph node involvement and thus it is a poor prognosis of breast cancer. We thought that c-erbB-2 may be a potential biologic marker for breast cancer prognosis and axillary lymph node involvement and may provide insight for c-erbB-2 expression and axillary involvement at the time of diagnosis for breast cancer in the future. In addition, in the current approach, there are studies suggesting that neoadjuvant systemic treatment can be given primarily in early-stage cancers and patients with axillary lymph node involvement. In this context, since the possibility of axillary lymph node involvement increases in c-erbB-2 positive patients, this should be taken into consideration during the treatment plan.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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